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INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference Case 21425		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/EP 03/10684	International filing date (day/month/year) 25.09.2003	Priority date (day/month/year) 27.09.2002	
International Patent Classification (IPC) or both national classification and IPC C12N15/53			
Applicant DSM IP ASSETS B.V. et al.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 1 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the International application</p>			
Date of submission of the demand 09.03.2004		Date of completion of this report 21.01.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized Officer Gurdjian, D Telephone No. +31 70 340-3388 	

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/EP 03/10684**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-20 as originally filed

Sequence listings part of the description, Pages

1-5 as originally filed

Claims, Numbers

1-3 received on 04.08.2004 with letter of 03.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
☒ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-3
	No: Claims	
Inventive step (IS)	Yes: Claims	1-3
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-3
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The present application relates to the cloning of the flavin adenine dinucleotide dependent D erythronate 4 phosphate dehydrogenase having the molecular data sequence with amino acid seq.id.2 and nucleotide seq.id.1 , from Sinorhizobium meliloti that is used for the recombinant vitamin B6 production .

Reference is made to the following documents:

- D1: CAPELA D; ET AL SWALL accession number Q92RK3
(1-12-2001) Putative oxidoreductase protein XP002266688
- D2: INTERPRO accession number IPR006094 FAD linked oxidase, N-terminal
(02-09-2002) XP002266689
- D3: PEASE ANDREW J ET AL: "Positive growth rate-dependent regulation of the pdxA, ksgA, and pdxB genes of Escherichia coli K-12." JOURNAL OF BACTERIOLOGY. UNITED STATES MAR 2002, vol. 184, no. 5, March 2002 (2002-03), pages 1359-1369, XP002266687 ISSN: 0021-9193

1. Novelty(Article 33.2 PCT)

D1 discloses a flavin adenine dinucleotide FAD linked oxidoreductase protein , that is the translation of the cloned chromosome sequence of Sinorhizobium meliloti and has 100.000% identity (100.000% ungapped) in 496 aa overlap (1-496:1-496) with seq.2 . D1 is translated from the EMBL sequence with accession number AL591785 that has 100.000% identity (100.000% ungapped) in 1491 nt overlap (1491-1:84766-86256) with seq.1 . The sequence of D1 comprises the IPR006094 domain that discloses the flavin adenine dinucleotide FAD linked oxidase, N-terminal , domain . (see D2) .

In view of the fact that the subject-matter of present claims 1-3 relates to a process of biological production of vitamin B6 , the subject-matter of claim 1-3 is new .

2. Inventive step(Article 33.3 PCT)

D3 discloses the positive growth rate-dependent regulation of the pdxB gene of Escherichia coli K-12. The pdxB gene encodes a erythronate-4-phosphate dehydrogenase that is involved in the production of vitamin B6 .(see the abstract , page

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1359 last line and page 1368 references 3,16,18,21,30,31,36,38,40,48)

A comparison of the amino acid sequence of reveals the no essential structural identity link between the PdxB of D3 and PdxR of the present application (Identity 11.0% , similarity 17.4%)

D3 is considered to be the closest prior art and differs form the present application by the provision an alternative vitamin B6 biosynthesis production pathway involving an alternative erythronate-4-phosphate dehydrogenase .

The problem to be solved is the provision of further vitamin B6 biosynthesis production method .

The person skilled in the art would have had the incentive to solve that problem, but he would NOT have a reasonable expectation of success of using the putative oxidoreductase protein of D1 , having only 11% identity with the erythronate-4-phosphate dehydrogenase of the present application and failing to disclose the erythronate-4-phosphate dehydrogenase function , in a process of RECOMBINANT biological production of vitamin B6 .

The subject matter of present claims 1-3 is hence inventive .

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Claims

1. A process for the biological production of vitamin B₆ which comprises cultivating a host cell transformed or transfected by an isolated DNA or by a vector or plasmid comprising the isolated DNA under the condition conducive to the production of vitamin B₆, and
5 recovering vitamin B₆ from the culture, wherein the isolated DNA comprises a nucleotide sequence that encodes PdxR, which is a flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase, selected from the group consisting of:
(a) a DNA sequence identified by SEQ ID NO:1 or the complementary strand thereof;
(b) a DNA sequence which hybridizes under standard conditions to the DNA sequence
10 complementary to the DNA sequence defined in (a) or a fragment thereof, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase;
(c) a DNA sequence which codes for a polypeptide having the amino acid sequence encoded by the DNA sequence of (a) or (b);
15 (d) a DNA sequence which is identical to the extent of at least 80% to a DNA which codes for a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase; and
(e) a DNA sequence which codes for a polypeptide which comprises an amino acid
20 sequence which is identical to the extent of at least 80% to the amino acid sequence of SEQ ID NO: 2, and encodes a polypeptide having the activity of flavin adenine dinucleotide-dependent D-erythronate 4-phosphate dehydrogenase.
2. A process for the biological production of vitamin B₆ which comprises introducing the isolated DNA as claimed in any one of (a) to (e) in claim 1 into an appropriate host cell,
25 cultivating the obtained host cell under the condition conducive to the production of vitamin B₆, and recovering vitamin B₆ from the culture.
3. The process according to claim 1 or 2, wherein said host cell belongs to the genus *Sinorhizobium*.

KJS / 30.07.2004

AMENDED SHEET